

What is claimed is:

1. A formulation for targeting an epitope on an antigen expressed in a mammal, the formulation comprising a pharmaceutically acceptable carrier in combination with,

a dimeric biosynthetic construct for binding at least one preselected antigen, the construct comprising:

- (a) two polypeptide chains, each of which have:
  - an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with a said preselected antigen, and

and a C-terminal tail having a non-self-associating structure under physiological conditions and comprising at least a crosslinking means, and

- (b) a linkage coupling said crosslinking means on said two polypeptide chains,

said dimeric construct having a conformation permitting binding of a said preselected antigen by the binding site of each said polypeptide chain when administered to said mammal.

2. A formulation for targeting an epitope on an antigen expressed in a mammal, the formulation comprising a pharmaceutically acceptable carrier in combination with,

a dimeric biosynthetic construct for binding preferentially to a preselected antigen, the construct comprising:

- (a) two polypeptide chains, each of which have:
  - an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with a said preselected antigen, and

a C-terminal tail having a non-self-associating structure under physiological conditions and comprising at least a crosslinking means, and

- (b) a linkage coupling said crosslinking means to form a homodimeric construct,

said homodimeric construct having a conformation permitting binding to said preselected antigen in said mammal with an avidity greater than the avidity of either of said polypeptide chains individually.

3. A polypeptide chain for binding preferentially to a preselected antigen, the polypeptide chain comprising:

an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with said preselected antigen, and

a C-terminal tail having a non-self-associating structure under physiological conditions and comprising at least a crosslinking means.

4. The polypeptide chain of claim 1, 2 or 3 wherein said C-terminal tail comprises the amino acid sequence Ser-Cys.

5. The polypeptide chain of claim 1, 2 or 3 wherein said C-terminal tail comprises the amino acid sequence (Gly)<sub>4</sub>-Cys.
6. The polypeptide chain of claim 1, 2 or 3 wherein said C-terminal tail comprises the amino acid sequence (His)<sub>6</sub>-(Gly)<sub>4</sub>-Cys.
7. The polypeptide chain of claim 1, 2 or 3 wherein said C-terminal tail can chelate one or more ions.
8. The polypeptide chain of claim 7 wherein said ion is a metal ion.
9. The polypeptide chain of claim 1, 2 or 3 wherein said crosslinking means is a derivatizable amino acid side chain.
10. The polypeptide chain of claim 9 wherein said derivatizable amino acid is selected from the group consisting of lysine, arginine and histidine.
11. The polypeptide chain of claim 9 wherein said derivatizable amino acid is a cysteine amino acid.
12. The polypeptide chain of claim 1, 2 or 3 wherein said crosslinking means comprises a posttranslationally modified amino acid.
13. The polypeptide chain of claim 12 wherein said posttranslationally modified amino acid is the

Asn residue located in the amino acid sequence selected form group of Asn-Xaa-Ser and Asn-Xaa-Thr.

14. The formulation of claim 1 or 2 wherein said linkage is a chemical bridge.
15. The formulation of claim 1 or 2 wherein said linkage comprises a disulfide bond.
16. The formulation of claim 1 or 2 wherein said linkage comprises a bismaleimido-hexane cross-linker.
17. The formulation of claim 1 or 2 wherein said linkage comprises a bismaleimidocaproyl amino acid linker.
18. The formulation of claim 1 or 2 wherein said linkage comprises a peptidyl linker.
19. The formulation of claim 1 or 2 wherein said linkage forms a substantially inflexible structure under physiological conditions.
20. The formulation of claim 1 or 2 wherein said linkage has a length and composition optimized for binding of two preselected antigens expressed on a tissue surface in a mammal.
21. The formulation of claim 1 or 2 wherein said linkage comprises a detectable moiety.
22. The formulation of claim 21 wherein said detectable moiety comprises Technetium<sup>-99m</sup>.

23. The formulation of claim 21 wherein said detectable moiety comprises means for inducing proton relaxation in vivo.
24. The formulation of claim 1 or 2 wherein said dimeric biosynthetic construct targets said epitope on said antigen with an avidity greater than that of a monoclonal antibody having the same antigenic determinant as said construct, or a fragment thereof.
25. The formulation of claim 1 or 2 wherein said dimeric biosynthetic construct targets said epitope on said antigen with an avidity greater than that of either of said polypeptide chains individually.
26. The formulation of claim 1 or 2 wherein said preselected antigen is expressed on the surface of a cell.
27. The formulation of claim 1 or 2 wherein said antigen is an intracellular component exposed upon cell lysis.
28. The formulation of claim 1 or 2 wherein said dimeric construct binds two different epitopes.
29. The formulation of claim 1 or 2 wherein one of said binding sites further comprises a catalytic site.

30. The formulation of claim 1 or 2 wherein one of said binding sites binds an epitope on a therapeutic agent to be targeted to a cell surface.
31. The formulation of claim 30 wherein said therapeutic agent is a cytotoxic agent.
32. The formulation of claim 1, 2 or 3 wherein said construct has improved in vivo imaging characteristics.
33. A single-chain Fv (sFv) polypeptide for binding preferentially to a c-erbB-2 or a c-erbB-2-related tumor antigen, the polypeptide comprising:

an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with said c-erbB-2 or c-erbB-2-related tumor antigen.
34. The polypeptide chain of claim 1, 2, 3 or 33 wherein said FR sequences are derived from a human immunoglobulin.

35. The polypeptide chain of claim 1, 2, 3 or 33 wherein said CDR sequences are derived from an immunoglobulin that binds c-erbB-2 or a c-erbB-2 related antigen.
36. The polypeptide chain of claim 35 wherein said CDR sequences are derived from an immunoglobulin selected from the group consisting of the monoclonal antibodies 520C9, 741F8, and 454C11.
37. The polypeptide chain of claim 1, 2, or 3 having the amino acid sequence found in SEQ ID NO. 1.
38. The polypeptide chain of claim 33 having the amino acid sequence defined by residues 1 through 245 in SEQ ID NO. 1.
39. The polypeptide chain of claim 1, 2, 3 or 33 further comprising a detectable moiety.
40. The polypeptide chain of claim 39 wherein said detectable moiety comprises a radioactive atom.
41. The polypeptide chain of claim 40 wherein said detectable moiety comprises Technetium<sup>-99m</sup>.
42. A DNA sequence encoding the polypeptide chain of claim 1, 2, 3 or 33.
43. A host cell transfected with a DNA of claim 42.



44. A method of imaging a preselected antigen in a mammal expressing said antigen, said method comprising the steps of:
- (a) administering to said mammal the formulation of claim 1 or 2 having affinity for said preselected antigen, at a concentration sufficient to permit extracorporeal detection of said construct bound to said preselected antigen; and
  - (b) detecting said polypeptide chain bound to said antigen.
45. The method of claim 44 for use in magnetic resonance imaging.
46. The formulation of claim 1 or 2 for use as an in vivo imaging agent.
47. The formation of claim 1 or 2 wherein said biosynthetic construct is capable of remaining localized to target tissue in a mammal for a longer time than either of said polypeptide chains individually.
48. A formulation for targeting an epitope on an antigen expressed in a mammal, the formulation comprising a pharmaceutically acceptable carrier in combination with,
- a dimeric biosynthetic construct for binding at least one preselected antigen, the construct comprising:

- (a) two polypeptide chains, each of which have: an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with a said preselected antigen, and

an N-terminal tail comprising at least a crosslinking means, and

- (b) a linkage coupling said crosslinking means on said two polypeptide chains, said dimeric construct having a conformation permitting binding of a said preselected antigen by the binding site of each said polypeptide chain when administered to said mammal.

49. A formulation for targeting an epitope on an antigen expressed in a mammal, the formulation comprising a pharmaceutically acceptable carrier in combination with,

a dimeric biosynthetic construct for binding at least one preselected antigen, the construct comprising:

- (a) two polypeptide chains, each of which have: an amino acid sequence defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain comprising complementarity determining regions (CDRs) interposed between framework regions (FRs), the CDRs and FRs of each polypeptide chain together defining a binding site immunologically reactive with a said preselected antigen,

said polypeptide linker further comprising a crosslinking means, and

- (b) a linkage coupling said crosslinking means on said two polypeptide chains, said dimeric construct having a conformation permitting binding of a said preselected antigen by the binding site of each said polypeptide chain when administered to said mammal.